

CHAPTER 1:

Overview of *Caenorhabditis elegans*

INTRODUCTION

In order to understand the nature of neuronal modulation, it is essential not only to know where to look in the neural network, but to know what to expect in the unaltered state. The advantage of early mapping and a small nervous system¹ is easily apparent, as characterization of functional circuits is both necessary and makes the following work possible. The nematode *Caenorhabditis elegans* with its 302 neurons and stereotyped connectivity is uniquely positioned for the study of state-dependent modulation, and the ease of handling and powerful genetics is an added bonus.

1.1 A BRIEF DESCRIPTION OF *C. ELEGANS*

C. elegans is a free-living roundworm in the Phylum *nematoda*. It lives in the soil-air interface and feeds on bacteria that grow on rotting fruit. *C. elegans* has several traits that make it a convenient and powerful tool for genetic analysis and behavioral testing. It grows from egg to adult in roughly three days and grows to 2-3mm in length (Figure 1). The adult hermaphrodite produces an average of two hundred progeny², and in the absence of male worms, these progeny are genetically identical plus or minus any spontaneous mutations. Considering the variability of behavior, minimal genetic variation is an advantage.

The body of *C. elegans* is an unsegmented, tapered cylinder whose structure is maintained by a tube-like arrangement of muscles attached to the hypodermis and a tough cuticle cover. It uses a series of opposing muscles to deform the cuticle and initiate locomotion. *C. elegans* crawls or swims through its environment with a characteristic sinusoidal pattern. Its trajectory is a random walk, and *C. elegans* decelerates in the presence of food or potential mates. In contrast, the frequency of reversal and directional change increases in the absence of food, ensuring that the worm will be likely to spend more time in nutrient-rich areas².

C. elegans is relatively easy to maintain and can be grown on media or agar plates with bacteria. It feeds by drawing the bacteria through its mouth and passing it to its pharynx. The pharynx consists of three large sets of muscles controlled by its own nervous system. It is organized into the anterior and posterior bulbs, which grinds and filters the bacteria into the intestine. The pharyngeal nervous system comprises of about 20 neurons, and autonomously controls the alimentary system. The somatic nervous system is made up of the remaining neurons.

1.2 *C. ELEGANS* NERVOUS SYSTEM

The *C. elegans* hermaphrodite nervous system comprises of 302 neurons. The male has an additional 79 neurons that are chiefly involved in the control of mating. The somatic nervous system is organized into ganglia in the head and tail (Figure 2a). The primary ganglia exists in the head of the worm (Figure 3), and the nerve ring is a synapse-rich band of processes that wraps around the pharynx anterior to the posterior bulb.

C. elegans uses chemosensation as its primary way to find food, avoid noxious conditions, find mates, and make appropriate decisions about development.² The main sensory ganglia, the amphid, phasmid, and labial neurons penetrate the cuticle to sense the external environment.³ There are 32 sensory neurons in this group, and their cilia are either directly or indirectly exposed by openings made by the socket and sheath cells³ (Figure 3b). These neurons exist in pairs, and proper development of the cilia are key to proper sensory neuron function.⁴ Early studies with laser ablation allowed identification of specific sensory neurons and stimuli to which they respond.

A majority of sensory neurons reside in the head and tail ganglia. The exception is mechanosensory neurons whose cell bodies and processes are in relative proximity to areas that they innervate. The motor neurons that control locomotion are studded along the ventral midline, and can be characterized as cholinergic motor neurons that promote muscle contraction and GABAergic motor neurons that promote muscle relaxation. The two nerve cords, dorsal and ventral, carry the processes between the head and tail ganglia. It is approximated that this neural network has 6400 chemical synapses, 1500 neuromuscular junctions, and 900 gap junctions.⁵

The connections of these neurons are stereotyped and show little or no variation in the wildtype N2 strain. The computational components of the nervous system can be organized roughly into four layers: the sensory layer that is innervated by external stimuli, the first layer of interneurons that receive sensory information, the second layer of command interneurons that process sensory and interneuron input and convert them to a motor repertoire that is carried out by the fourth layer of motor neurons.⁶ Although these circuits are fixed anatomically, modulation of the function of these circuits occurs through the use of neurotransmitters and neuromodulators, such as dopamine, serotonin, and acetylcholine.^{7,8}

Despite its simple nervous system, *C. elegans* exhibits a wide range of behaviors.⁹ In addition to more simple motor tasks, such as locomotion, pharyngeal contraction, and egg-laying, it is able to carry out complicated motor repertoires, such as mating and escape response. A worm

will exhibit preferences to certain temperature, chemical concentration, mechanosensory environment, and absence or presence of other worms. The worm can respond to a variety of negative cues and will show quick avoidance behavior in response to harsh mechanical stimulation and aversive chemicals.² *C. elegans* shows a type of associative learning, and when placed in a thermal, chemical, or electrical gradient, it migrates consistently and robustly towards zones associated with conditions previously associated with food or unstarved conditions.^{10,11} Furthermore, basic habituation and paired conditioning are observable and are modified by the presence of food.^{12,13}

1.3 SIGNAL TRANSDUCTION IN NEURONS

The *C. elegans* candidate chemoreceptors are related to the family I (rhodopsin-related) G-protein coupled receptors. Well-conserved GPCRS also encode receptors that recognize serotonin, acetylcholine, and neuropeptides, but these genes are general and are not expressed preferentially in the chemosensory neurons.² It is conjectured that individual sensory neurons would express a unique set of GPCRs that would bind to specific ligands and confer their functional identities. There are over a thousand predicted GPCRs, and few genes have been identified in the function of specific sensory neurons.

The ASH nociceptive sensory neuron is one of best studied, and it is a polymodal sensory neuron that responds to mechanical stimuli as well as to metals and changes in osmolarity. Many amphid sensory neurons, in addition to the ASH, signal through transient receptor potential (TRP) channels encoded by *osm-9*¹⁴ and *ocr-2*¹⁵ genes (Figure 4). The TRP channel superfamily encodes vertebrate channels that sense osmosensation, pain, and pressure². It is believed that these channels are downstream of the *odr-3* GPCR signaling and require synthesis of long-chain polyunsaturated fatty acids (PUFAs).² Loss of *odr-3* and *gpa-3* diminish calcium transients that are mediated by the TRP channels *osm-9* and *ocr-2* in the ASH.¹⁶

1.4 FUNCTIONAL CIRCUITS in *C. ELEGANS*

The existence of static connectivity diagrams reconstructed from electron microscopy data is an immense advantage in any systems approach to *C. elegans* neurobiology. Analyzing these maps have helped to pinpoint specific cells and pathways for genetic and behavioral analyses and to identify motifs of connectivity.^{17,18} Early studies have heavily relied upon mutant analysis and

targeted elimination of specific circuit components using laser ablation.¹⁹ Although many of these studies indicate the necessity of both circuit and molecular components for specific behaviors, functional understanding was greatly improved by high-resolution data on the real-time processing done by these neurons.

Many functional circuits have been heavily studied over the last two decades. Of these, the best characterized are the avoidance circuit, the control of locomotion, and sensory coding.²⁰ The ease of studying these circuits is obvious. The avoidance circuit is the most direct and simple of all circuits in this simple system. It consists of a handful of sensory neurons directly connected to the command interneurons¹⁶ and is easily observable as an immediate response. The control of locomotion is measurable and the role of various genes and neurons in the control of amplitude, speed and frequency of distinct features has been noted and characterized.²¹ However, mechanical stimuli are often manual, and precise consistent stimulation of the same area with the same pressure is not trivial. Therefore, many chemosensory neurons and their downstream components have been heavily studied with respect to their activity in response to each other and in the presence of modulating factors.

1.5 *C. ELEGANS* IS A GOOD CANDIDATE FOR STATE-SPECIFIC MODULATION

The choice of circuit was key for my study of state-specific modulation, and there were multiple criteria in my choice: the exhibition of state-dependent change in behavior, characterization of the functional circuit that drives behavior, availability of or ability to make tools to manipulate the circuit across layers of processing, and simplicity of circuit. The avoidance circuit was the clear winner. Unlike chemosensory neurons mediating attraction, it has direct connection to the command interneurons and no obvious additional computational processing.¹ Decreased sensory arousal in response to noxious cues had been observed²², and the primary avoidance mediating neuron, the ASH^{8,16}, as well as the downstream command interneurons^{23,24} and motor neurons²⁵ had already been characterized in the adult worm.

FIGURES

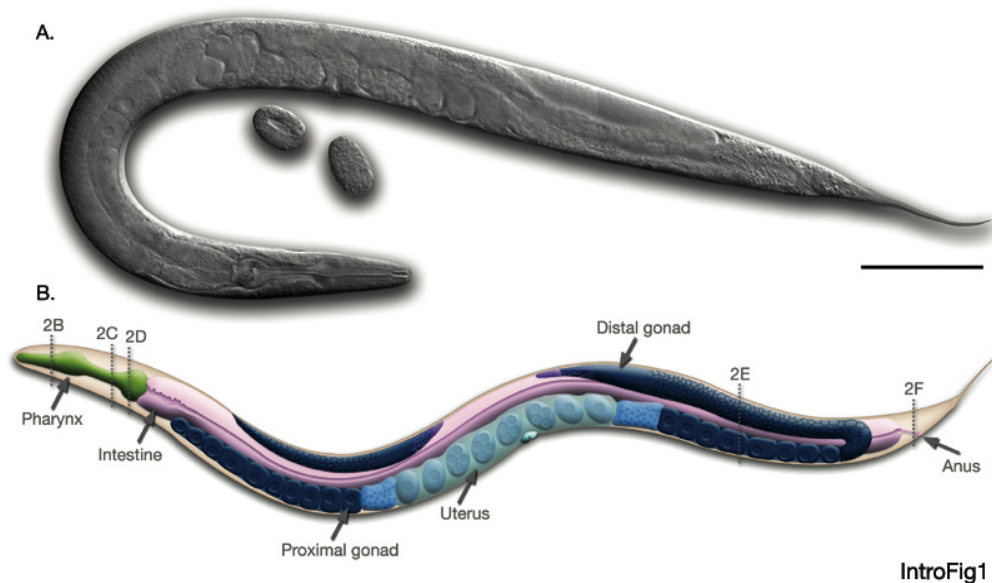


Figure 1. Anatomy of the Adult Hermaphrodite. A. DIC image of the adult hermaphrodite. Scale bar is 1mm B. Schematic drawing of the anatomical structures. (Unmodified figure from Wormatlas.²⁶)

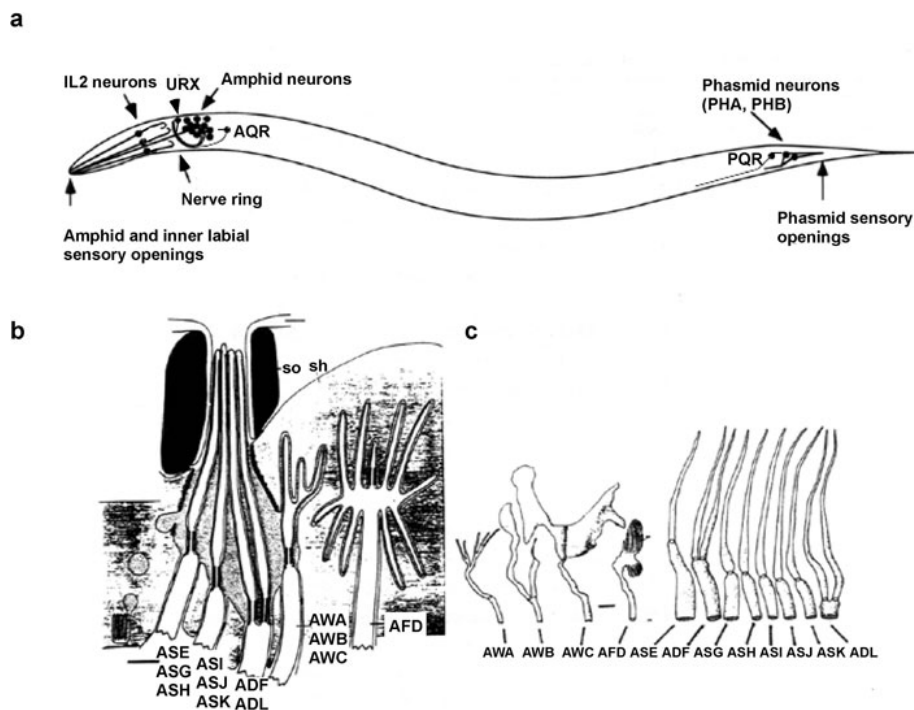


Figure 2. Structure of chemosensory organs. a. Distribution of chemosensory neurons in the animal. Amphids contain 12 associated chemosensory or thermosensory neurons. Phasmids contain two chemosensory neurons, PHA and PHB. Each inner labial organ contains one IL2 chemosensory and one IL1 mechanosensory neuron. There are two URX neurons, one AQR neuron, and one PQR neuron. b. Detailed structure of the amphid sensory opening showing the socket (so), sheath (sh), and ciliated nerve endings. c. Detailed structure of the cilia in the 12 classes of amphid neurons. (Unmodified from Wormbook.²⁷)

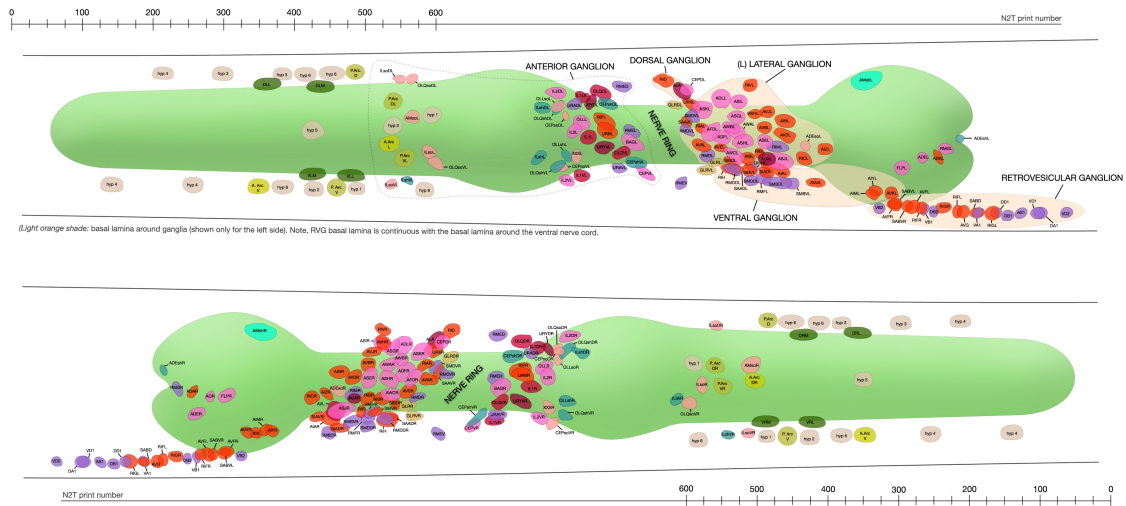


Figure 3. *C. elegans* head neurons. Schematic drawing of all head neurons in the left and right sides of the worm with respect to the pharyngeal muscle drawn in green. Note the ganglia drawn in beige in the top panel. (Unmodified figure from Wormatlas.²⁶)

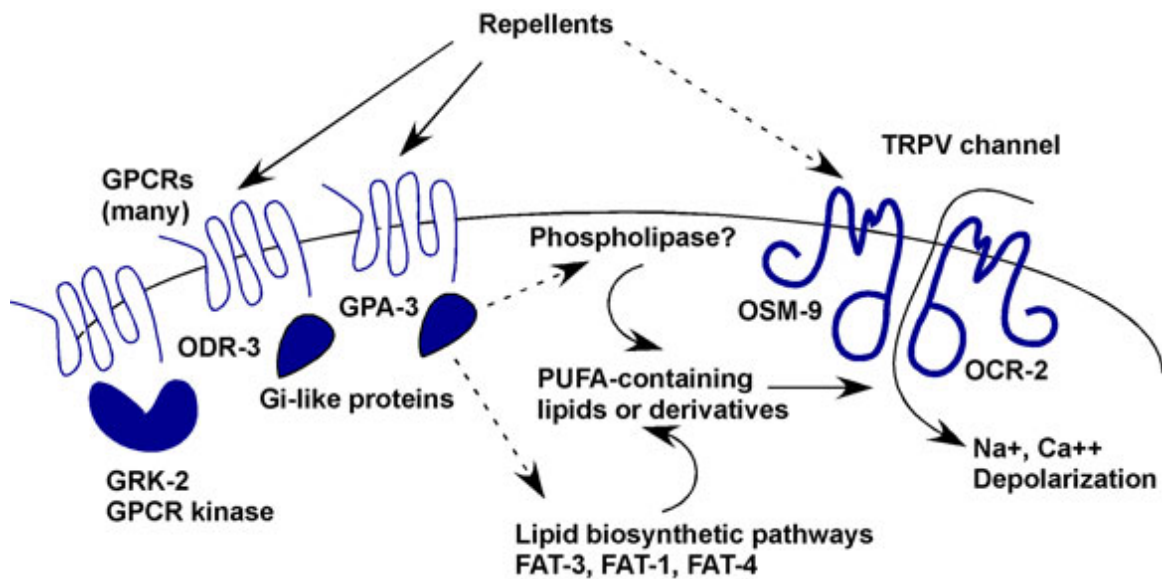


Figure 4. Potential signal transduction pathway for nociception in ASH cilia. A likely model is that repellents are detected by GPCRs and possibly by other molecules such as ion channels. GPCRs activate the Gi-like proteins ODR-3 and GPA-3, which regulate the production or consumption of phospholipids containing PUFAs (omega-3 and omega-6 polyunsaturated fatty acids). The GPCR kinase GRK-2 also promotes ASH activation. Lipid mobilization opens the TRPV channels encoded by OSM-9 and OCR-2 to depolarize the cell. TRPV channels may also be directly activated by mechanical, chemical or osmotic stimuli, perhaps with the assistance of accessory subunits such as OSM-10. (Unmodified figure from Wormbook.²⁷)

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